Optimization in the Identification, Selection and Induction of Maturation of Subtypes of Cardiomyocytes derived from Human Embryonic Stem Cells

Grant Award Details

Optimization in the Identification, Selection and Induction of Maturation of Subtypes of Cardiomyocytes derived from Human Embryonic Stem Cells

Grant Type: Tools and Technologies I

Grant Number: RT1-01143

Investigator:

Name:	Patrick McDonough
Institution:	Vala Sciences, Inc.
Type:	PI

Disease Focus: Heart Disease

Human Stem Cell Use: Embryonic Stem Cell

Cell Line Generation: Embryonic Stem Cell

Award Value: $870,717

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

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Grant Application Details

Application Title: Optimization in the Identification, Selection and Induction of Maturation of Subtypes of Cardiomyocytes derived from Human Embryonic Stem Cells

Public Abstract: Cardiovascular diseases remain the major cause of death in the western world. Stem and progenitor cell-derived cardiomyocytes (SPC-CMs) hold great promise for the myocardial repair. However, most of SPC-CMs displayed heterogeneous and immature electrophysiological phenotypes with substantial automaticity. Implanting these electrically immature and inhomogeneous CMs to the hearts would be arrhythmogenic and deleterious. Further optimization in identification, selection and inducing maturation of subtypes of CMs from primitive SPC-CMs are paramount for developing a safe and effective cell-based therapy. Commonly used CM isolation techniques are microdissection, density sedimentation or promoter-driven, fluorescence-activated cell sorting (FACS). Microdissection and density sedimentation are labor intensive and lack of purity. Promoter-driven FACS may compromise cell viability and which promoter is proficient for selection remains unclear. We have established several antibiotics (Abx)-resistant human embryonic stem cell (hESC) lines conferred by lentiviral vectors under the control of various cardiac-specific promoters. With simple Abx treatment, we have easily isolated >95% pure hESC-CMs at various stages of differentiation from embryoid bodies (EBs). Using this Abx selection system, we also found that electrical maturation and differentiation of primitive hESC-CMs depended heavily on developmental cues from extracardiac cells in the EBs. This Abx selection system therefore could be used easily to purify CMs for mechanistic studies and future cell-based therapies. However, the subtype specification of atrial, ventricular and pacemaking CMs appears to occur at very early stages of differentiation because early EBs possess all three types of cells. Furthermore, various cardio-specific promoters have been shown to select preferentially certain subtypes of CMs. In order to use these promoters and Abx resistance to sub-select particular types of CMs at early stages of differentiation, we need to know the timing and sequence of expressions of various cardiac promoters during the EB development. For this later purpose, we will generate hESC lines expressing different colors of fluorescent proteins under the control of various cardiac-specific promoters respectively to determine the timing of expressions of these promoters in the EBs. Based on the sequence of expression, we will generate the Abx-resistant hESC lines under the control of these promoters to sub-select CMs. We will then study the EP properties of these sub-selected hESC-CMs and their interactions with extra-cardiac cells. The overall goal of this proposal is to establish an In Vitro system to track the sequence of expressions of various promoters in order to sub-select particular phenotypes of CMs by the Abx-resistance method. As a result, we will be able to optimize the selection and induction of a population of mature and homogeneous hESC-CMs for a safe and effective cell-based therapy.

Statement of Benefit to California:	Cardiovascular diseases remain the major cause of death in the western world. Stem and progenitor cell (SPC)-based cell therapies in animal and human studies suggest promising therapeutic potentials. However, most SPC-derived cardiomyocytes (SPC-CMs) displayed heterogeneous and immature electrophysiological (EP) phenotypes with substantial automaticity. Implanting these electrically immature and inhomogeneous CMs to the hearts would be arrhythmogenic and deleterious. Further optimization in identification, selection and inducing maturation of subtypes of CMs from these primitive SPC-CMs are badly needed. Most frequently used isolation techniques are microdissection, density sedimentation or promoter-driven, fluorescence-activated cell sorting (FACS). Microdissection and density sedimentation are labor intensive and lack of purity. Promoter-driven FACS may compromise cell viability and which promoter is proficient for the cardiomyocyte selection remains to be determined. None of the laboratories in the world has success in developing an easy and efficient way to isolate the SPC-CMs. As a result, no method has been developed to induce the maturation of SPC-CMs. We already have the technology to efficiently isolate pure populations of human embryonic stem cell-derived CMs (hESC-CMs) from the embryoid bodies. The proposed research will further determine which type of promoter is best to properly sub-select a specific phenotype of hESC-CMs for future cell-based therapies in California. Most importantly, using this antibiotics-based selection method, we have started investigating the methods for inducing maturation of these sub-selected and primitive CMs. With both goals achieved, we will make California the first state to have a safe and effective cell-based therapy for myocardial repair with a mature and homogeneous population of hESC-CMs. None of stem cell-related research in California is devoted to optimize the selection, identification and induction of maturation of a specific phenotype of hESC-CMs in order to develop a safe cell-based therapy. The proposed research will be the first to achieve this goal proposed by CIRM Tools and Technologies Award. The success of this proposal will also make California the epicenter of the next generation of cell therapies and will benefit its citizens who have significant cardiovascular diseases.